ability of ZRA to inhibit ice recrystallization and to reduce ice crystal growth rate imply on its tremendous potential usages in the field of cryopreservation and other biological and engineering fields.

Supported by ISF, ERC and IRG.

Conflict of interest: None declared.

E-mail address: braslavs@agri.huji.ac.il (I. Braslavsky)

http://dx.doi.org/10.1016/j.cryobiol.2012.07.049

---

49. Interactions between water and triacylglycerols may explain faster aging rates in stored germplasm at low temperatures. Aline Schneider Teixeira [1], Daniel Ballesteros [2], Antonio Diego Molina-Garcia [1], Christina Walters [2], [1] ICTAN (CSIC), Jose Antonio Novais 10, 28040 Madrid, Spain, [2] USDA-ARS-National Center for Genetic Resources Preservation, Fort Collins, CO 80521, USA

Longevity of orthodox (desiccation tolerant) seeds, pollen and fern spores characteristically increases with decreasing storage temperature. We have discovered numerous seed and fern spore species that age *faster* when stored in the freezer near −20 °C compared to storage at higher or lower temperatures. Seeds exhibiting this temperature anomaly are considered to be intermediate between orthodox and recalcitrant physiologies. Intermediate traits are often associated with high proportions of saturated fatty acids within the triacylglycerols (TAG), accumulated into cells as food reserves. TAG high in saturated fatty acids crystallize and melt within the temperature range of the observed longevity anomalies and we have hypothesized a link between TAG phase change and increased damage to germplasm cells. Damage is exacerbated as water content within stored seeds and fern spores increases from near 5% to about 12%. These observations have led us to hypothesize that damage results from an interaction between TAG, water, temperature and time. The purpose of this research is to explore the extent and significance of possible interacting factors on the viability of stored germplasm. Our work begins with characterizing the kinetics of TAG and water phase changes in peanut (*Arachis hypogaea*) and papaya (*Carica papaya*) seeds equilibrated to different water contents and stored at temperatures between −5 and − 80°C. Water and TAG phase was measured using a Perkin Elmer Differential Scanning Calorimeter. Cytoplasm ultra-structure was visualized without chemical fixatives using low temperature scanning electron microscopy (cryo-SEM) performed with a Zeiss DSN 960 scanning microscope equipped with a Cryotrans CT-1500 cold plate (Oxford, UK). The time dependency of phase changes was assessed over a 3 week period. We report that TAG crystallization in seeds is independent of water content and occurs over several days. The amount of water that freezes in seeds increases with storage duration in a manner consistent with TAG crystallization kinetics.

Conflict of interest: None declared.

Source of funding: This work was funded from project "CRYODYMINT" (AGL2010-21989-C02-02), Spanish "*Plan Nacional I + D+i 2008–2011*", M.E.C. and ARS National Center for Genetic Resources Preservation. A.S. Teixeira was supported by the CSIC, within the JAE-Pre program, partially funded from the European Social Fund.

E-mail address: teixeiraline@hotmail.com (A.S. Teixeira)

http://dx.doi.org/10.1016/j.cryobiol.2012.07.050

---

50. Relevance of cell biophysical behaviour and membrane fluidity for explaining freezing resistance of lactic acid bacteria. Stéphanie Passot [1], Marielle Bouix [1], Julie Gautier [2], Pascale Lieben [2], Stéphanie Cenard [2], Sarrah Ghorbal [1], Fernanda Fonseca [2], [1] AgroParisTech, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78 850 Thiverval-Grignon, France, [2] INRA, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78 850 Thiverval-Grignon, France

The cell membrane is the primary site for freezing injury. During cooling, ice forms outside the cell resulting in an increase of the solutes concentration in the extracellular medium. This cryo-concentration of solutes induces water transport from the intracellular medium of cell to the extracellular medium and thus cell dehydration and cell injury. Cell membrane permeability to water is influenced by its fluidity property which is governed by the composition and the structure of the lipid bilayer. By changing the composition of the medium, the composition and organisation of the lipid bilayer of the cell membrane will be modified resulting in increasing or decreasing freezing resistance. Furthermore, another important phenomenon occurs during cooling of cell suspensions leading to the modification of membrane fluidity: the lipid phase transition from a liquid crystalline phase to a gel phase. The liquid crystalline state is characterized by increased lipid head group spacing, increased disorder in the lipid acyl chains, and a small bilayer thickness. In the gel phase the lipid head groups are very tightly packed, the lipid acyl chains become straighter and ordered, and the bilayer thickness increases.

Our objective is to gain a better understanding of the relationships between membrane fluidity, lipid phase transition, membrane water permeability and freezing resistance of lactic acid bacteria.

Two suspensions of *Lactobacillus bulgaricus CFL1* were produced using either MRS broth or mild whey based culture media. The freezing resistance of both bacterial populations protected with sucrose was evaluated by measuring the cultivability and acidification activity of bacteria before and after freezing at −80 °C. The membrane lipid phase behaviour was studied *in situ* using Fourier transform infrared spectroscopy during freezing and thawing. Membrane fluidity was evaluated with fluorescence polarization using a lipophilic fluorescent probe embedded in the membrane, namely the DPH. The fluorescence measurements were performed on a flow cytometer at various temperatures. Bacterial cell dehydration was investigated by submitting cells to osmotic stress by increasing the concentration on a non penetrating solute (sucrose). The changes in cell volume with time were measured by Coulter techniques and the biophysical parameters of water transport were estimated.

Bacterial population grown in MRS broth exhibited a better resistance to freezing than bacteria grown in mild whey. This better resistance can be ascribed to a lower lipid phase transition temperature and higher membrane fluidity. The detailed analysis of the results is in progress and will enable clarification of the relationships between freezing resistance, membrane fluidity and water permeability.

Conflict of interest: None declared.

Source of funding: None declared.

E-mail address: spassot@grignon.inra.fr (S. Passot)

http://dx.doi.org/10.1016/j.cryobiol.2012.07.051

Anthony J. Reardon received the John K. Critser Travel Award

51. A comparative assessment of mitochondria and membrane cryobiological responses of HUVEC to low temperatures. Anthony J. Reardon [1], Janet A. W. Elliott [2], Locksley E. McGann [1], [1] Departments of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada, [2] Departments of Laboratory Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, Canada

The extent of cellular cryoinjury incurred during cryopreservation protocols is traditionally assessed with membrane integrity assays, often used as an upper limit in determining viability. However, exposure of cells to subzero temperatures and conditions may lead to changes in various aspects of cellular structure and function that would not be considered in studies that rely on membrane integrity assessments alone. Slower cooling rates in particular have been shown to adversely affect the metabolism (McGann et al., 1988; and Sherman, 1972) and structural properties (Tchir et al., 2010) of mitochondria in addition to the occurrence of damage to the plasma membrane during freeze–thaw stress. The objective of the study was to use a mitochondrial assay to investigate a secondary aspect of cell response to directly compare with a traditional assessment of membrane integrity under identical conditions. An interrupted slow cooling method was used to examine the occurrence of damage from slow cooling to intermediate subzero experimental temperatures, and damage during rapid cooling when plunged into liquid nitrogen. Human umbilical vein endothelial cells (HUVECs) were cooled at 0.2 °C/min to temperatures between −3 °C and −40 °C in the absence of cryoprotectant. Samples were then either thawed directly in a 37 °C water bath or plunged into liquid nitrogen before thawing. The membrane integrity of cells was determined using a combination of the nucleic fluorescent dyes Syto13 and ethidium bromide, and mitochondrial polarization was indicated with the cationic carbocyanine dye JC-1. The number of cells determined to be membrane intact and the number of cells containing polarized mitochondria decreased with decreasing temperature in directly-thawed samples. Depolarized mitochondria was found in 50% of cells at −15 °C, whereas 50% of cells were membrane compromised at a lower temperature (−30 °C). HUVEC plunged into liquid nitrogen from experimental temperatures showed an increase in the number of membrane intact cells with decreasing experimental temperature to a maximum of 40% at −20 °C, but <8% of these cells contained polarized mitochondria. Directly thawed samples showed a greater proportion of cells with depolarized mitochondria at higher subzero temperatures than cells with membrane damage, indicating that subcellular changes occur in cells under conditions that do not compromise the integrity of the plasma membrane. At some experimental temperatures, plunged HUVEC showed significant membrane integrity, immediately post-thaw, but few of these membrane intact cells contained polarized mitochondria. This demonstrates that damage to organelles and the plasma membrane may occur